Quantitative Structure Activity Relationship Studies of Sulfamide Derivatives as Carbonic Anhydrase Inhibitor: As Antiglaucoma Agents

Surendra Kumar[1], Vineet Singh[2] and Meena Tiwari[2,*]

[1]*Faculty of Pharmacy, Northern India Engineering College, Akhilesh Das Nagar, Sector-II, Chinhut, Lucknow, India;* [2]*Molecular Modeling Lab, Department of Medicinal and Pharmaceutical Chemsitry, Shri Govindram Seksaria Institute of Pharmaceutical Sciences, 23 Park Road, Indore – 452003, Madhya Pradesh, India*

Abstract: Selective inhibition of Ciliary process enzyme i.e. Carbonic Anhydrase-II is an excellent approach in reducing elevated intraocular pressure, thus treating glaucoma. Due to characteristic physicochemical properties of sulphonamide (Inhibition of Carbonic Anhydrase), they are clinically effective against glaucoma. But the non-specificity of sulphonamide derivatives to isozyme, leads to a range of side effects. Presently, the absence of comparative studies related to the binding of the sulphonamides as inhibitors to CA isozymes limits their use. In this paper we have represented "Three Dimensional Quantitative Structure Activity Relationship" study to characterize structural features of Sulfamide derivative [$RR'NSO_2NH_2$] as inhibitors, that are required for selective binding of carbonic anhydrase isozymes (CAI and CAII). In the analysis, stepwise multiple linear regression was performed using physiochemical parameters as independent variable and CA-I and CA-II inhibitory activity as dependent variable, respectively. The best multiparametric QSAR model obtained for CA-I inhibitory activity shows good statistical significance (r= 0.9714) and predictability (Q^2=0.8921), involving the Electronic descriptors viz. Highest Occupied Molecular Orbital, Lowest Unoccupied Molecular Orbital and Steric descriptors viz. Principal moment of Inertia at X axis. Similarly, CA-II inhibitory activity also shows good statistical significance (r=0.9644) and predictability (Q^2=0.8699) involving aforementioned descriptors. The predictive power of the model was successfully tested externally using a set of six compounds as test set for CA-I inhibitory activity and a set of seven compounds in case of CA-II inhibitory activity with good predictive squared correlation coefficient, r^2_{pred}=0.6016 and 0.7662, respectively. Overview of analysis favours substituents with high electronegativity and less bulk at R and R' positions of the parent nucleus, provides a basis to design new Sulfamide derivatives possessing potent and selective carbonic anhydrase-II inhibitory activity.

Key Words: QSAR, carbonic anhydrase enzyme, sulfamide derivative, highest occupied molecular orbital, anticancer drugs, antiviral drugs, carbonic anhydrase inhibitors, austin model-1.

INTRODUCTION

Carbonic anhydrases (**CAs**) are wide spread and well characterized metalloenzymes, containing a zinc ion per polypeptide chain (MW ~30 kDa), present in *Archaea*, *prokaryotes and eukaryotes,* being encoded by three distinct, evolutionarily unrelated gene families: the ***α-CAs*** (present in *vertebrates, eubacteria, algae* and cytoplasm of green plants), the ***β-CAs*** (predominantly in *eubacteria, algae* and chloroplasts of both mono- as well as dicotyledons) and the ***γ-CAs*** (mainly in *Archaea* and some *eubacteria*). At least 14 different CA isozymes or CA-related proteins (CARP) with very different subcellular localization and tissue distribution were described in higher vertebrates. Basically, there are several cytosolic forms (CA I-III, CA VII), four membrane-bound isozymes (CA IV, CA IX, CA XII and CA XIV), one mitochondrial form (CA V) as well as a secreted CA isozyme (CA VI), where these enzymes play crucial physiological roles [1-6]. Actually, these enzymes catalyse a very simple physiological reaction, the interconversion of carbon dioxide to bicarbonate ion. That is why, CA enzymes are also involved in physiological processes related to respiration (transport of CO_2/bicarbonate between metabolizing tissues and the lungs), pH and CO_2 homeostasis, electrolyte secretion in a variety of tissues/organs, biosynthetic reactions (such as the gluconeogenesis, lipogenesis and ureagenesis), bone resorption, calcification, tumorigenicity [7,8]. The presence of these ubiquitous enzymes in many tissues and many different isoforms represents an attractive target for the design of inhibitors or activators with biomedical applications [9]. Potent, clinically used inhibitors of CAs are acetazolamide (**AAZ**), methazolamide (**MZA**), and ethoxzolamide (**EZA**), dichlorphenamide (**DCP**), dorzolamide (**DZA**), and brinzolamide (**BRZ**), all having general formula RSO_2NH_2, where R is generally an aromatic, aliphatic or heterocyclic moiety. The terminal sulphonamide group which must have the amide nitrogen unsubstituted slip into the active site and chelate to the zinc atom, displacing the normally associated water ligand and preventing ingress of the carbondioxide molecule [10,11]. These sulphonamides are widely used clinically, mainly as antiglaucoma agents, but successful exploitation of sulphonamides led to the serial development of improved antibiotics, insulin-releasing hypoglycaemic drugs, antihypertensive drugs, antiviral drugs and anticancer drugs etc.

Recently Sulfamide derivatives with general formula (NH_2SO_2NH2), in which terminal amino group were substituted with a variety of aromatic/heterocyclic compounds

*Address correspondence to this author at the Molecular Modeling Lab, Department of Medicinal and Pharmaceutical Chemsitry, Shri Govindram Seksaria Institute of Pharmaceutical Sciences, 23 Park Road, Indore – 452003, Madhya Pradesh, India; Tel: +91-731-2564636;
E-mail: mtiwari@sgsits.ac.in

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having strong affinity for the carbonic anhydrase active site resulted in improvement in Carbonic anhydrase inhibitory activity. Further, X-ray Crystallographic structure of sufamide bound to the physiologically relevant isozyme hCA II has been reported and it was critically assumed from the study of the findings that the sulfamide binds to the zinc ion of the enzyme (which is situated at the bottom of a 15 Å deep active site cleft) *via* its deprotonated amide group (Zn-N distance of 1.70 Å), and this nitrogen also donates H-bond to Oγ of Thr 199 *via* its remaining hydrogen. This suggested that Oγ acts as an acceptor for the NH group of the inhibitor, but hydroxyl group of Thr 199 donates H-bond to one of the terminal carboxylate oxygens of the adjacent Glu 106. Involving its second oxygen, this carboxylate forms H-Bond network as acceptor to the backbone NH of Arg 246 and *via* a water moleule to the hydroxyl oxygen of Tyr 7. These interactions enhance the nucleophilicity of Zinc-bound water molecule and orient the substrate (CO_2) in a favourable location for the nucleophilic attack, leading to formation of bicarbonate coordinated to Zn (II). The bicarbonate ion is then displaced by a water molecule and liberated into solution. In order to regenerate the zinc-hydroxide species of the enzyme (active species), a proton transfer reaction takes place. This extended extarcoordination results in a distorted tetrahedral arrangement around the metal ion, the remaining three ligands of Zinc being His94, His96, His119. The second oxygen of the SO_2 group of the inhibitor was shown to be close to the backbone NH of Thr199 (2.81 Å), which accordingly accept a H-bond from Thr199NH. This oxygen replaces the 'deep water' in the ligand-free-enzyme. Finally, the second, uncharged NH_2 group of Sulfamide forms a H-bond of 2.90 Å to an adjacent water molecule, which in turn is involved in H-bond network with three other water molecules present in the binding pocket. In addition, Sulfamide nitrogen forms a second weak hydrogen bond to the OH group of Thr 200 (3.20 Å) and a third contact has been found to another water molecle (3.26 Å). In short, this very simple inhibitor, present as the negatively charged $(NH)SO_2NH_2^-$ ion, shows a large number of favorable contacts in the binding pocket of Carbonic Anhydrase. In Sulfamide there is an additional S-N bond, which suggest that these moieties are able to participate in an intricate three new H-bonding network that differes entirely from the one formed by the aromatic/heterocyclic sulfonamides [12].

The quantitative structure-activity relationship (QSAR) study is a useful tool for rational search of bioactive compounds. QSAR studies have predictability and simultaneously provide deeper insight into the mechanism of drug receptor interactions [13]. We, therefore, report here a QSAR study on Sulfamide derivatives for their carbonic anhydrase inhibitory activity to explore the physicochemical properties desirable for inhibition and thus, may be helpful in designing new molecules that are predicted to exhibit enhanced biological activity [14-16].

MATERIALS AND METHODS

Biological Data

A data set consisting of 26 Sulfamide derivatives reported by Casini *et al.* [17] (Fig. (**1**)) with inhibitory activity on carbonic anhydrase I and carbonic anhydrase II was selected for the present study. Carbonic anhydrase I and Carbonic anhydrase II inhibitory activity is represented in *Ki*, which is Michaelis inhibition constant (nM) representing the affinity of the substrate to carbonic anhydrase enzyme. The Inhibition constant (Ki) was converted to free energy values using the $\Delta G = -RT \ln K$ for the regression studies [18]. The molecular structures of these compounds are shown in Table **1, 2**. Four compounds from carbonic anhydrase I inhibitory activity and three compounds from carbonic anhydrase II inhibitory activity were exempted for molecular modeling studies as they were showing undefined biological activity. The data set was divided into training set of 16 compounds and test set of 6 compounds for hCA I (human Carbonic Anhydarse I) and similarly for hCA II (human Carbonic Anhydarse II), training set of 16 compounds and test set of 7 compounds using random selection method.

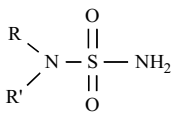

Fig. (1). Structure of Sulfamide analogs used in the present study.

Calculation of Structural Properties for QSAR Analysis

Molecular modeling studies were carried out using CS Chem Office software version 6.0 (Cambridge soft) [19]. Molecular Structures of all the compounds in training and test set were sketched using Chem Draw and copied to Chem 3D ultra version 6.0 to create 3D structure. Each 3D molecular structure was subjected to energy minimization technique using Allinger's Molecular Mechanics (MM_2) force field followed by geometry optimization using semiempirical Qunatum Mechanics AM-1 (Austin Model-1) [20-23]. Hamiltonian approximations method and closed shell (restricted) wave function available in MOPAC module by fixing root mean square (RMS) gradient as 0.1 and 0.0001 kcal/mol·Å respectively was used for calculating partial atomic charges and electron density on various atoms. Charges were kept as mulliken and maximum number of iteration was set to 10000. The lowest energy structure was used for each molecule to calculate Molecular descriptors (electronic, spatial and thermodynamic) using "compute properties module" of Chem 3D ultra version 6.0 (Table **3**).

Multiple Linear Regression Analysis

Multiple Linear Regression Analysis was performed by in-house built Computer program VALSTAT [24]. The autocorrelated parameters were eliminated depending on their individual correlation with the biological activity in order to avoid simple collinearity problem. All possible combinations of parameters were considered for the QSAR study. The predictive power of the models was validated by Leave-One-Out (LOO) cross-validation method [25, 26]. The selected QSAR model was tested by prediction of the activity of test set compounds. Predicted residual sum of square (PRESS), cross-validated squared correlation coefficient (Q^2), and standard deviation error of prediction (SDEP) were considered for validation of these models. The results from cross-validated analysis were expressed as the cross-validated squared correlation coefficient (Q^2). The Q^2 is defined as,

Table 1. Structures and Activities of Sulfamide Derivatives Used in Training Set

C. No.	Substituents		[a]hCA I(nM)		[a]hCA II(nM)	
	R	R'	[b]Ki	pKi[c]	[b]Ki	pKi[c]
S-1	n-Bu	H	173	-2.2380	148	-2.1702
S-2	Cyclohexyl	H	164	-2.2148	450	-2.6532
S-5	$PhCH_2$	H	133	-2.1238	123	-2.0899
S-6	$-(CH_2)_5$	H	155	-2.1903	148	-2.1702
S-7	$-(CH_2)_6$	H	163	-2.2121	131	-2.1367
S-8	Ph	H	13	-1.1139	12	-1.0791
S-9	$4\text{-}CH_3\text{-}C_6H_4$	H	15	-1.1760	13	-1.1139
S-10	$4\text{-}Cl\text{-}C_6H_4$	H	19	-1.2787	15	-1.1761
S-11	$4\text{-}Br\text{-}C_6H_4$	H	23	-1.3617	21	-1.3222
S-12	$4\text{-}I\text{-}C_6H_4$	H	18	-1.2552	17	-1.2304
S-14	$4\text{-}MeO\text{-}C_6H_4$	H	14	-1.1461	11	-1.0413
S-15	$4\text{-}OH\text{-}C_6H_4$	H	16	-1.2041	12	-1.0791
S-16	$4\text{-}NO_2\text{-}C_6H_4$	H	18	-1.2552	13	-1.1139
S-17	$4\text{-}EtO_2C\text{-}C_6H_4$	H	26	-1.4149	19	-1.2787
S-18	$4\text{-}NC\text{-}C_6H_4$	H	20	-1.3010	16	-1.2041
S-19	$4\text{-}Me_2N\text{-}C_6H_4$	H	17	-1.2304	21	-1.3222

Abbreviations: human Carbonic Anhydrase (hCA); [a]determined by esterase method; [b]representing the binding affinity of substrate to enzyme; [c]negative logarithm of Ki.

$$Q^2 = 1 - \sum(Y_{pred} - Y_{act})^2 / \sum (Y_{act} - Y_{mean})^2$$

Where Y_{pred}, Y_{act} and Y_{mean} are predicted, actual and mean values of the target property (-log IC50) respectively. $\sum(Y_{pred} - Y_{act})^2$ is the Predictive Residual Error Sum of Squares (PRESS).

PRESS is an important cross-validation parameter as it is a good approximation of the real predictive error of the models. To further access the robustness and statistical confidence of the derived models, bootstrapping analysis was performed (25 runs). The r^2_{bs} is average squared correlation coefficient calculated during the validation procedure which is computed from a subset of variables used one at a time for the validation procedure. Following statistical parameters were considered to compare the generated QSAR models: correlation coefficient (*r*), standard error of estimate (s), variance ratio (F) at specified degree of freedom, 99.9% confidence intervals of the regression coefficients.

Table 2. Structures and Activities of Sulfamide Derivatives Used in Test Set

C. No.	Substituents		[a]hCA I (nM)		[a]hCA II (nM)	
	R	R'	[b]Ki	pKi[c]	[b]Ki	pKi[c]
S-3	2-Adamantyl	H	960	-2.9822	890	-2.9493
S-4	$PhCH_2$	$PhCH_2$	>100,000	-	647	-2.8109
S-13	$4\text{-}CF_3\text{-}C_6H_4$	H	8	-0.9030	7	-0.8450
S-20	C_6F_5	H	34	-1.5314	32	-1.5051
S-21	$3\text{-Benzoyl-}C_6H_4$	H	62	-1.7923	49	-1.6901
S-22	2-Naphthyl	H	39	-1.5910	36	-1.5563
S-23	$(CH_2)_2\text{-SS-}(CH_2)_2$	-	149	-2.1731	27	-1.4313

Abbreviations: human Carbonic Anhydrase (hCA); [a]determined by esterase method; [b]representing the binding affinity of substrate to enzyme; [c]negative logarithm of Ki.

Table 3. List of Descriptors Used in this Study

S.No	Descriptors	Type	Description (Units)
1	BP	Thermodynamic	Boiling Point (Kelvin)
2	CP	Thermodynamic	Critical Pressure (Kelvin)
3	CT	Thermodynamic	Critical Temperature (bar)
4	HF	Thermodynamic	Heat of formation (Kcal/mole)
5	HLC	Thermodynamic	Henrys Law Constant
6	IGTC	Thermodynamic	Ideal Gas Thermal Capacity
7	Log P	Thermodynamic	Logarithmic Partition Coefficient
8	MP	Thermodynamic	Melting Point (Kelvin)
9	MR	Thermodynamic	Molar Refractivity (cm^3/mole)
10	SGP	Thermodynamic	Standard Gibb's Free Energy (kJ/mole)
11	VDW	Thermodynamic	Van der Waals Force (kcal/mole)
12	PC	Thermodynamic	Partition Coefficient For Water / Octanol
13	NVDW	Thermodynamic	Non-1,4-Van der Waals Force (kcal/mole)
14	SE	Thermodynamic	Stretch Energy (kcal/mole)
15	SBE	Thermodynamic	Stretch Bend Energy (kcal/mole)
16	TORE	Thermodynamic	Torsion Energy (kcal/mole)
17	E_T	Thermodynamic	Total Energy (kcal/mole)
18	CAA	Steric	Connolly Accessible Surface Area (Å)
19	CMA	Steric	Connolly Molecular Surface Area (Å)
20	CSEV	Steric	Connolly Solvent- Excluded Volume (Å)
21	EM	Steric	Exact Mass (g/mole)
22	MW	Steric	Molecular Weight (atomic mass units)
23	OVAL	Steric	Ovality
24	PMI-X	Steric	Principal Moments of Inertia at x axis (g/moles Å)
25	PMI-Y	Steric	Principal Moments of Inertia at y axis (g/moles Å)
26	PMI-Z	Steric	Principal Moments of Inertia at z axis (g/moles Å)
27	DIPOLE-X	Electronic	Dipole Moment-X axis (Debye)
28	DIPOLE-Y	Electronic	Dipole Moment-Y axis (Debye)
29	DIPOLE-Z	Electronic	Dipole Moment-Z axis (Debye)
30	EE	Electronic	Electronic Energy (Ev)
31	E_{HOMO}	Electronic	Energy of Highest Occupied Molecular Orbital (eV)
32	E_{LUMO}	Electronic	Energy of Lowest Unoccupied Molecular Orbital (eV)
33	RE	Electronic	Repulsion Energy (eV)
34	BE	Electronic	Bending Energy (kcal/mole)
35	DDE	Electronic	Dipole-Dipole Energy (kcal/mole)

RESULTS AND DISCUSSION

The training set of sulfamide derivatives was subjected to multiple linear regression analysis using molecular descriptors as independent parameters and Carbonic anhydrase I inhibitory activity and Carbonic anhydrase II inhibitory activity as dependent parameters. Among the several models generated, the best model was selected for each biological activity for further discussion on the basis of correlation coefficient (r>0.9), Standard deviation (S<0.3), Fischer value> 99.9%, Cross-validated squared correlation coefficient (Q^2 >0.3) and boot strapping r^2 (r^2_{bs}).

QSAR Analysis for Carbonic Anhydrase-I Inhibitory Activity

Model-2 (Table **4**) which showed better predictability for Carbonic anhydrase I inhibitory activity was selected for further discussion.

Model-2 showed good correlation (r=0.9625) between parameters viz. Highest Occupied Molecular Orbital Energy (E_{HOMO}), Lowest Unoccupied Molecular Orbital Energy (E_{LUMO}) and Principal Moment of Inertia at X axis (PMI-X) and experimental pKi of inhibitors. The r^2 value of 0.9265 (Fig. (**2**), Table **5**) corresponds to a confidence limit superior at 99.9%, which minimizes the risk of chance correlation. The standard deviation (0.1393) implicates the acceptability of the model. Predictive power of the model was further ascertained by cross-validation test, which is significant (Q^2 = 0.8952) (Fig. (**3**), Table **5**). The boot strapping r^2 (0.9444), values reflect the accuracy of the model. The model has small error value (SDEP= 0.1440 and S_{PRESS}= 0.1663) representing its predictability. The validity of the model was checked using the test set of six compounds which were not included in the model development.The model has good predictive squared correlation coefficient (0.6016), which is in agreementwith the accepted criteria. (Fig. (**4**), Table **6**). The correlation matrix between parameters shows orthogonal nature of parameter as desired in QSAR analysis (Table **7**).

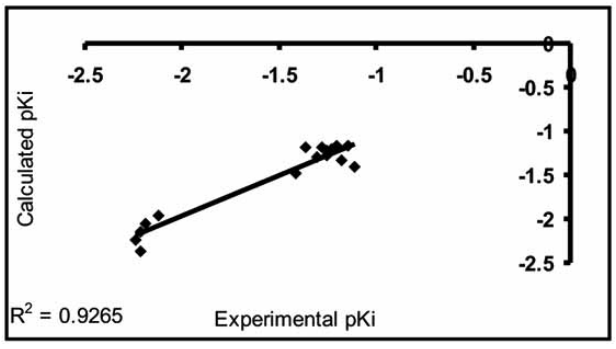

Fig. **(2)**. Experimental pKi versus Calculated pKi of Training set for human Carbonic Anhydrase-I Enzyme-Inhibitor Complex (Model-2).

QSAR Analysis for Carbonic Anhydrase II Inhibitory Activity

Several models were generated for carbonic anhydrase II activity. Two models were selected based on the statistical and validation parameters for further discussion (Table **4**). Model-3 shows better results based on the statistical and validation parameters viz. S, F-test, Q^2, SDEP, S_{PRESS} and r^2_{pred}, and is statistically more significant, having better predictability for Carbonic anhydrase II inhibitory activity.

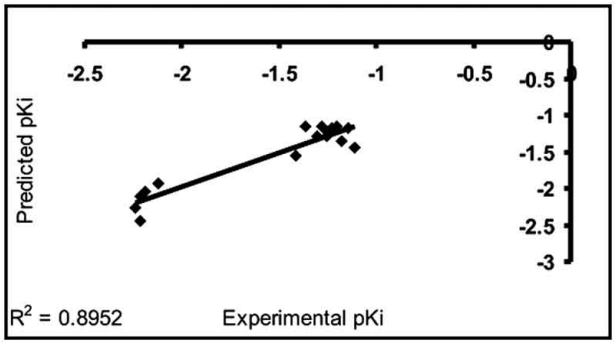

Fig. **(3)**. Experimental pKi versus Predicted pKi (LOO) of Training set for human Carbonic Anhydrase-I Enzyme-Inhibitor Complex (Model-2).

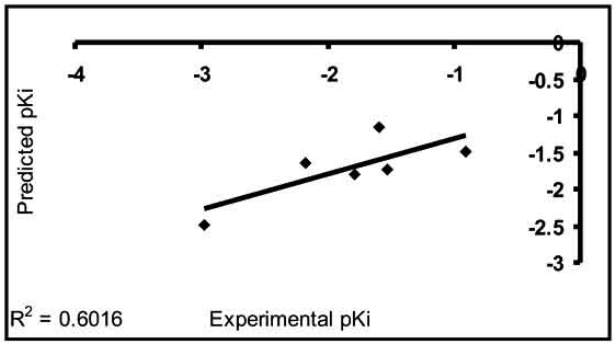

Fig. **(4)**. Experimental pKi versus Predicted pKi (LOO) of Test set for human Carbonic Anhydrase-I Enzyme-Inhibitor Complex (Model-2).

Model-3 explain 93.02% variance (Fig. (**5**)) having predictabilty for 86.99% in carbonic anhydrase II inhibitory activity. In this model, E_{HOMO} contributes positively, whereas E_{LUMO} and PMI-X contributes negatively for activity. The standard deviation is low (0.1561), which shows the actual quality of fit for model.

To ascertain the predictivity of the model, leave-one-out cross validation process, boot strapping technique and randomization test were performed. The significant values Q^2 (0.8724, Fig. (**6**), Table **5**), S_{PRESS} (0.2132), SDEP (0.1867), bootstrapping r^2 (0.9269) and chance correlation < 0.001 implicates that results were not based on chance. The validity of the model was also checked externally using the test set of seven compounds, which were not included in the model development. The model shows a good external predictability (r^2_{pred} = 0.7662, Fig. (**7**), Table **6**). The correlation matrix shows non collinear nature of discriptors as desirable in the QSAR analysis (Table **7**).

Table 4. Summary of Multiple Linear Regression Analysis with Statistical and Validation Parameters

QSAR Model			Statistical parameters					Validation parameters			
			n	r	r^2	S	F	Q^2	r^2bs	SDEP	S_{PRESS}
hCA-I	Model-1	pKi = [2.6911(±1.1075)] + E_{HOMO} [0.5005 (±0.1112)] + E_{LUMO} [-0.8299 (±0.2725)] + NVDW [-0.0378 (±0.0313)]	16	0.9714	0.9436	0.1219	67.0453	0.8921	0.9520	0.1461	0.1687
	Model-2	pKi = [2.6265(±1.2956)] + E_{HOMO} [0.4548 (±0.1182)] + E_{LUMO} [-0.7881 (±0.3071)] + PMI-X [-0.0014 (±0.0020)]	16	0.9625	0.9264	0.1393	50.4040	0.8952	0.9444	0.1440	0.1663
hCA-II	Model-3	pKi = [2.4909(±1.4516)] + E_{HOMO} [0.4819 (±0.1325)] + E_{LUMO} [-1.0320 (±0.3441)] + PMI-X [-0.0022 (±0.0022)]	16	0.9644	0.9302	0.1561	53.3471	0.8699	0.9269	0.1847	0.2132
	Model-4	pKi = 3.1137 [(±1.6949)] + E_{HOMO} [0.4731 (±0.1404)] + E_{LUMO} [-0.9739 (±0.3651)] +CSEV [-0.0052 (±0.0067)]	16	0.9600	0.9216	0.1655	47.0310	0.8425	0.9289	0.2031	0.2346

QSAR Model Abbreviations: Correlation Coefficient (r), Squared Correlation Coefficient (r^2), Standard deviation (S), Fischer Value (F) at specified degree of freedom (Limit of confidence, 99.9%), Leave-one-out cross validation coefficient (Q^2), boot strapping r^2 (r^2bs), standard deviation error of prediction (SDEP), Predictive Residual Error Sum of Squares (S_{PRESS}).

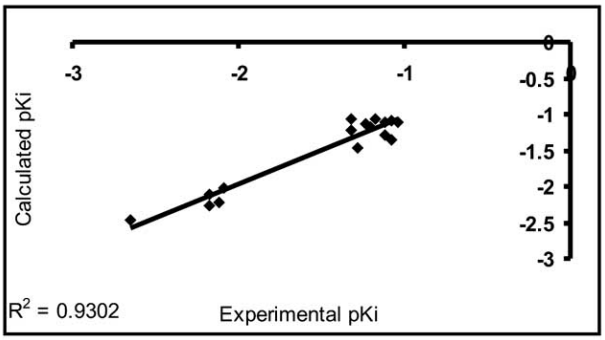

Fig. (5). Experimental pKi versus Calculated pKi of Training set for human Carbonic Anhydrase-II Enzyme-Inhibitor complex (Model-3).

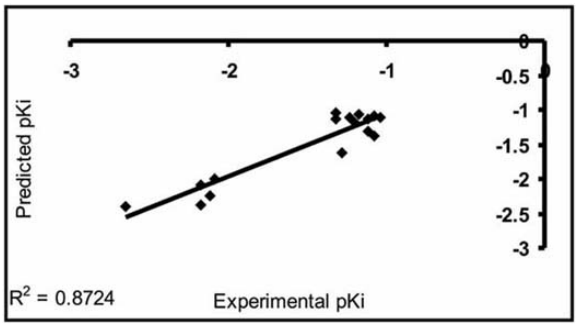

Fig. (6). Experimental pKi versus Predicted pKi (LOO) of Training set for human Carbonic Anhydrase-II Enzyme-Inhibitor Complex (Model-3).

Results of our study points out several issues which are important in the design consideration of selective new carbonic anhydrase inhibitor. It is evident from the selected best model Highest Occupied Molecular Orbital (E_{HOMO}) energy contributes positively whereas Lowest Unoccupied Molecular Orbital energy (E_{LUMO}) and Principal moment of Inertia along X axis (PMI-X) contribute negatively for carbonic anhydrase I and carbonic anhydrase II inhibitory activity. The importance of Electronic parameters describes non-covalent binding interaction between drug molecule and receptor. E_{HOMO} obtained by molecular orbital calculation, relates to the presence of high electron density, so molecules with high E_{HOMO} are able to donate their electron in charge transfer phenomena. X-ray crystallographic studies also show electron withdrawing substituents on aromatic/heterocyclic ring enhaces its H-bond network with COOH group of Glu 69. The positive coefficient with E_{HOMO} brings out the fact that substituents with high electronegativity at R or R' position might be favourable for binding of inhibitors with carbonic anhydrase I and II isozymes. This is in line with evidence that it is the anionic form $(NH)SO_2NH_2^-$, which is effective in holding the sulfamide to the positively charged Zn atom and inhibit the Carbon dioxide hydration activity of CA either by displacing a coordinated water or by expansion of the coordination sphere. The sulfamido group is oriented such that oxygen of Thr 199 forms a H-bond and this position allows one of the oxygens of the sulfonamido group to occupy a more distant fifth coordination site on the Zn according to *Kannan et al.* [27]. In this configuration *para*-substituents fall on a large slightly concave hydrophilic sur-

Table 5. Calculated and Predicted pKi for Training Set

C. No.	hCA I for Model-2		hCA II for Model-3	
	Cal. pKi	[a]Pred. pKi	Cal. pKi	[a]Pred. pKi
S-1	-2.2476	-2.2595	-2.2635	-2.3794
S-2	-2.3728	-2.4469	-2.4742	-2.3903
S-5	-2.0591	-2.0348	-2.1016	-2.0889
S-6	-2.1429	-2.1218	-2.2186	-2.2496
S-7	-1.9603	-1.9274	-2.0215	-2.0078
S-8	-1.4055	-1.4389	-1.3497	-1.3807
S-9	-1.3322	-1.3532	-1.2818	-1.3044
S-10	-1.1782	-1.1634	-1.0718	-1.0564
S-11	-1.1847	-1.1587	-1.0748	-1.0385
S-12	-1.2279	-1.2243	-1.1253	-1.1117
S-14	-1.1672	-1.1720	-1.1057	-1.1204
S-15	-1.1688	-1.1621	-1.0941	-1.0970
S-16	-1.2766	-1.2973	-1.1202	-1.1263
S-17	-1.4858	-1.5522	-1.4584	-1.6265
S-18	-1.2998	-1.2996	-1.1791	-1.1735
S-19	-1.2063	-1.1846	-1.2205	-1.1292

Abbreviations: Cal. pKi (Calculated pKi); Pred. pKi (Predicted pKi), [a]determined from leave one out method.

face, where one would expect them to be somewhat more than 50% desolvated if only simple partitioning is involved in binding. The E_{LUMO} is directly related to the electron affinity and characterizes the susceptibility of the molecule towards attack by nucleophiles [28]. It is also a measure of ionization potential and charge transfer phenomenon taking place in drug receptor interaction. The negative correlation of this term with activity indicates that lowering in E_{LUMO} value would result in increase in inhibitory activity of Sulfamide derivatives.

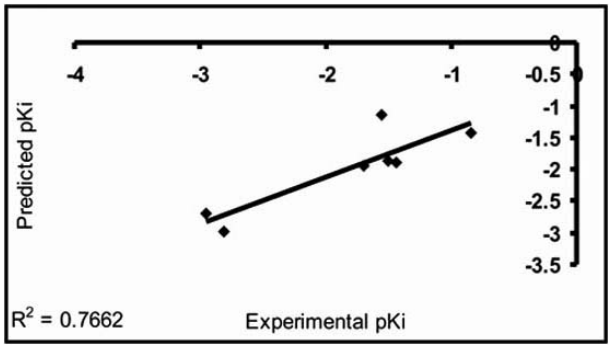

Fig. (7). Experimental pKi versus Predicted pKi (LOO) of Test set for human Carbonic Anhydrase-II Enzyme-Inhibitor Complex (Model-3).

In majority of cases Electronic parameters appear to produce their effect by substitution of electron withdrawing group, in the data set (compound no. **S-8, S-10, S-12** and **S-13**). It is evident from Table **1** that compound no. **S-8** is having more activity with phenyl ring at R position of the Sulfamide analogs in Training set whereas in the same data set compound no. **S-2** having saturated phenyl ring with least activity. Reducing or enlarging the ring at R position has least inhibitory activity shown by compound no. **S-6**. The Principal Moment of Inertia along X axis (PMI-X) is the quantified steric feature of drug molecules required for its complimentary fit with the receptor. It describes the orientation and distribution of total molecular mass in three dimensional space important for activity. Negative contribution of this parameter with activity indicates that less bulky substituents with proper orientation along X aixs at R or R' positions are easily incorporated in the enzyme cavity to interact with the binding site to inhibit its function. In data set, Compound no. **S-1** having large alkyl chain and compound no. S-3, 2-adamantyl substituted compound, were the most ineffective, because of their bulky nature and difficulty to accommodate in the enzyme active site. These finding further suggest that the less bulky substituents with high electronegative group at R or R' positions are important for inhibition of carbonic anhydrase enzyme. Predictive ability from the selected best model, model-3 shows high r^2_{pred} (0.7662> 0.6016) value, can be considered as the model to decribe somewhat more selectivity for carbonic anhydrase-II inhibi-

Table 6. Experimental and Predicted pKi for Test Set

C. No.	hCA I for Model-2		hCA II for Model-3	
	Exp. pKi	Pred. pKi	Exp. pKI	Pred. pKi
S-3	-2.9822	-2.4797	-2.9493	-2.6946
S-4	-	-	-2.8109	-2.9837
S-13	-0.9030	-1.4889	-0.8450	-1.4314
S-20	-1.5314	-1.7410	-1.5051	-1.8553
S-21	-1.7923	-1.8032	-1.6901	-1.9439
S-22	-1.5910	-1.1656	-1.5563	-1.1307
S-23	-2.1731	-1.6394	-1.4313	-1.8897

Abbreviations: Exp. pKi (Experimental pKi); Pred. pKi (Predicted pKi).

tory activity as compared to carbonic anhydrase-I inhibitory activity.

Table 7. Correlation Matrix of Parameters Significant for hCA I and hCA II Activity

	$^{a}E_{HOMO}$	$^{b}E_{LUMO}$	^{c}PMI-X
$^{a}E_{HOMO}$	1.0000		
$^{b}E_{LUMO}$	0.0775	1.0000	
^{c}PMI-X	0.1094	0.3335	1.0000

QSAR parameters abbreviations: [a]Highest Occupied Molecular Orbital Energy, [b]Lowest Unoccupied Molecular Orbital Energy, [c]Principal Moment of Inertia at X-axis.

Overview of Hansch Analysis

On the basis of the results discussed above, since electronic parameters viz; E_{HOMO}: 84.1144%; E_{LUMO}: 9.428% are contributing positively to the activity it can be concluded that the electron withdrawing substituents enhance the biological activity. The charge transfer phenomenon taking place in a drug receptor interaction upon binding was found to play a major role of Sulfamide analogs as carbonic anhydrase inhibitor. Also, the Steric parameters PMI-X: 6.4617%, shows negative contribution suggesting less bulkier substituents favors the biological activity.

From the above findings it can be suggested that electronic and steric properties have to be optimised while designing potent and selective carbonic anhydrase-II inhibitors.

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